UNIVERSITI PUTRA MALAYSIA

SOMATIC EMBRYOGENESIS IN MUSA SPP.

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FP 2002 18

SOMATIC EMBRYOGENESIS IN *MUSA* SPP.

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of Requirement for the Degree of Doctor of Philosophy

April 2002

Dedicated to

Departed soul of my grand-father
Hj. M. Mohi uddin Khan

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of requirement for the degree of Doctor of Philosophy

SOMATIC EMBRYOGENESIS IN *MUSA* SPP.

By

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April 2002

Chairman : Dr. Maheran Abdul Aziz

Faculty : Agriculture

Embryogenesis competent material (scalp) was initiated from shoot tip of *Musa* spp. cultivar Mas (AA), Berangan (AAA), Intan (AAA), Raja (AAB) and Tanduk (AAB). Somatic embryogenesis were investigated from four explant sources viz., scalps, male flower primordia, *in vitro* corm slices and immature ovules of *Musa acuminata* cv. Mas. Scalp formation was optimal on Murashige and Skoog (MS) medium with modified vitamins supplemented with 100 μM BAP and 1.0 μM IAA. Among the cultivars investigated, cv. Mas was the most responsive for scalp formation whereby 40% of the shoot tips formed scalps by the 7^{th} month of culture. Cultivar Mas was also the most responsive for meristematic globule formation from scalps attaining 100% meristematic globule formation by week 7 of culture of scalps in Z medium. Cells with embryogenic potential were released from the meristematic globules of cv. Mas after 10 to 12 months of culture of the meristematic globules in Z medium. The embryogenic cell suspension was transferred to liquid S medium and formed globular embryos after 3 to 4 months in culture. Matured globular embryos upon transfer to liquid S regeneration medium supplemented with 0, 1.0, 5.0, 10, 20, 40 and 80 μM BAP germinated to form roots but without shoots. Typical bi-polar

structure with prominent shoot and root poles was detected through a longitudinal section of a germinating somatic embryo.

In male flower primordia, 60 ± 7.07% of the cultured explants initiated callus after 3 months on MS medium supplemented with 5.7 μM IAA, 18.0 μM 2,4-D, 5.4 μM NAA and 4.0 μM biotin. Improved callus growth was observed on a reduced 2,4-D concentration of 4.5 μM with 5.7 μM IAA, 5.4 μM NAA and 4.0 μM biotin after 4 months of culture. Somatic embryo formation was observed in culture after 1 month on MS medium supplemented with 4.7 μM ABA. On transfer of the somatic embryo into germination medium containing MS/SH salts supplemented with 1.0 μM NAA, 0.5 μM kinetin, 0.2 μM zeatin, 2.0 μM BAP, 4.0 μM biotin, 100 mg/l glutamine, 100 mg/l malt extract and 45 g/l sucrose only plumule development occurred while root formation was not observed even after 1 month of culture.

Embryogenic callus formation from *in vitro* corm slices was observed in two media type, which were MS medium with modified vitamins supplemented with 0.5 μM 2,4-D and MS medium with modified vitamins supplemented with 5.0 μM 2,4-D, 1.0 μM proline, 100 mg/l casein hydrolysate and 40 mg/l cystein-HCl. Seventy percent of the cultured explants formed embryogenic callus at week 18 of culture. Embryogenic callus from the first medium formed root-like structures upon transfer to regeneration medium containing MS salts supplemented with 5.0 μM, 10 μM, 20 μM and 30 μM BAP. Embryogenic callus from the second medium also formed root-like structures on transfer to regeneration medium containing liquid

½ strength MS salts supplemented with 5.0 µM, 10 µM, 20 µM, 40 µM, 60 µM and 80 µM BAP.

Immature ovule explants responded to form vitreous callus instead of embryogenic callus in all the treatments tested. Among the five cultivars and four explant sources investigated, scalps and male flower-primordia of cultivar Mas could be considered promising for the induction of somatic embryogenesis.

Anatomical study of the shoot tip of banana cv. Mas (AA) indicated a conical-shaped structure consisting of several layers of leaf primordia covering the meristem apex. Shoot-bud proliferation which were of axillary origin and induced due to the inclusion of high cytokinin especially BAP in the medium were seen at the leaf bases of the shoot tips. Anatomical investigation of the meristematic globules indicated single cells originating from the starch riched cells in the peripheral layer of the meristematic globules.

Transformation study showed 9 cm target distance along with helium pressure of 1100 and 1350 psi to be the efficient variables for the transformation of scalps of cv. Mas whereby 40% of the scalps were transformed. A target distance of 6 cm along with helium pressure of 900 psi was optimal for the transformation of embryogenic cell suspension whereby 70% of the bombarded samples were transformed.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenunhi keperluan untuk ijazah Doktor Falsafah

EMBRIOGENESIS SOMA BAGI *MUSA* SPP.

Oleh

MD. HUMAYUN KABIR

April 2002

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'Scalp' iaitu struktur yang berkeupayaan menjadi embryogenik telah dijanakan dari mercu pucuk spesies *Musa* kultivar Mas (AA), Berangan (AAA), Intan (AAA), Raja (AAB) dan Tanduk (AAB). Embryogenesis soma daripada 4 sumber eksplan telah dikaji iaitu 'scalp' primodia bunga jantan, potongan umbisi daripada kultur *in vitro* dan ovul yang belum matang dalam *Musa* spp. kultivar Mas (AA). Pembentukan 'scalp' adalah optima pada media Murashige dan Skoog (MS) yang mengandungi vitamin terubah suai, 100 μM BAP dan 1.0 μM IAA. Di antara kultivar yang dikaji, kultivar Mas menunjukkan respon yang terbaik dari segi pembentukan 'scalp' dengan 40% mercu pucuk membentuk 'scalp' pada bulan ke 7 pengkulturan di dalam medium Z. Kultivar Mas juga didapati menunjukkan respon yang terbaik terhadap pembentukan dari 'scalp' dengan penghasilan 100% globul meristematik pada minggu ke 7 'scalp' dikultur di dalam medium Z. Sel dengan potensi embriogenik telah diperolehi daripada globul meristematik kultivar Mas selepas 10 ke 12 bulan globul meristematik dikultur di dalam medium Z. Sel ampaian embriogenik telah dipindahkan ke medium S dan membentuk embrio

globular selepas 3 ke 4 bulan dikultur. Embrio globular yang matang selepas dipindahkan ke media regenerasi yang mengandungi cecair S dengan 0, 1.0, 5.0, 10, 20, 40 dan 80 μM BAP bercambah membentuk akar tetapi tanpa pucuk. Struktur bi-polar yang tipikal dengan pucuk dan akar yang menonjol keluar telah dikenalpasti melalui keratan memanjang embrio somatik yang sedang cambah.

Bagi primodia bunga jantan, 60 ± 7.07 % daripada eksplan yang dikultur mula membentuk kalus selepas 3 bulan di dalam media MS yang dibekalkan dengan 5.7 μM IAA, 18.0 μM 2,4-D, 5.4 μM NAA dan 4.0 μM biotin. Pertumbuhan kalus yang lebih baik diperolehi dengan pengurangan kepekatan 2,4-D kepada 4.5 μM serta mengandungi 5.7 μM IAA, 5.4 μM NAA dan 4.0 μM biotin selepas 4 bulan dikultur. Pembentukan embriogenesis soma telah diperolehi selepas 1 bulan pengkulturan pada medium MS mengandungi 4.7 μM ABA. Apabila embrio soma tersebut dipindahkan ke dalam media percambahan yang mengandungi garam MS/SH yang dibekalkan dengan 1.0 μM NAA, 0.5 μM Kinetin, 0.2 μM Zeatin, 2.0 μM BAP, 4.0 μM biotin. 100mg/l glutamine, 100 mg/l ekstrak malt dan 45 g/l sucrose, hanya perkembangan pucuk berlaku sementara pembentukan akar tidak diperolehi selepas 1 bulan dikultur.

Pembentukan kalus embriogenik daripada potongan umbisi kultur *in vitro* telah diperolehi di dalam 2 media iaitu media MS dengan vitamin yang dimodifikasi yang dibekalkan dengan 0.5 μM 2,4-D dan media MS yang dibekalkan dengan 5.0 μM 2,4-D, 1.0 μM prolin, 100 mg/l kasein hidrolisat dan 40 mg/l sistein-HCl. Tujuh puluh peratus daripada eksplan yang dikultur membentuk kalus embriogenik

selepas 18 minggu dikultur. Kalus embriogenik daripada media pertama membentuk struktur seperti akar apabila dialihkan ke media regenerasi yang mengandungi garam MS yang dibekalkan dengan 5.0 μM, 10 μM, 20 μM dan 30 μM BAP sementara kalus embriogenik daripada media kedua membentuk struktur seperti pucuk atau akar apabila dialihkan ke media regenerasi yang mengandungi ½ garam MS cecair yang dibekalkan dengan 5.0 μM, 10 μM, 20 μM, 40 μM, 60 μM dan 80 μM BAP.

Eksplan daripada ovul yang belum matang membentuk kalus yang bersifat 'vitreous' dan tidak membentuk kalus embriogenik di dalam semua rawatan yang diuji. Di antara lima kultivar dan 4 sumber eksplan yang dikaji, eksplan 'scalp' dan primodia bunga jantan daripada kultivar Mas boleh di anggap berpotensi untuk membentuk embriogenesis soma.

Kajian anatomi ke atas mercu pucuk pisang kultivar Mas (AA) menunjukkan struktur berbentuk kon yang terdiri daripada beberapa lapisan primodia daun yang menutupi meristem apeks. Tunas baru yang berproliferasi yang berasal daripada tunas aksil dan teransang akibat penambahan sitokinin yang tinggi khususnya BAP ke dalam media telah dilihat pada pangkal daun mercu pucuk tersebut. Penyelidikan anatomi ke atas globul meristematik, menunjukkan sel tunggal muncul daripada sel yang kaya dengan kanji di dalam lapisan persisian globuls meristematik tersebut.

Kajian transformasi menunjukkan jarak sasaran 9 cm dengan tekanan helium pada 1100 dan 1350 psi merupakan pemboleh ubah yang efisien untuk transformasi 'scalp' kultivar Mas dengan 40% daripada 'scalp' tersebut mengalami transformasi.

Jarak sasaran 6 cm dengan tekanan 900 psi adalah optima bagi transformasi sel ampaian embriogenik dengan 70% daripadanya berjaya ditransformasikan.

ACKNOWLEDGEMENTS

First of all, I thank almighty Allah for giving me the strength and ability to complete this study.

I wish to express my deepest appreciation and sincere thanks to Dr. Maheran Abdul Aziz, the chairman of the Supervisory Committee, for her kind support, guidance, understanding, constructive criticism, suggestions and invaluable advice, throughout the duration of this study and the preparation of this thesis.

I would like to express my sincere thanks to my committee members, Dr. Mihdzar Abdul Kadir, Prof. Dr. Marziah Mahmood and Dr. Suhaimi Napis, for their creative suggestions, necessary corrections and co-operation in completing this thesis.

I am also indebted to Prof. Rahman Abdul Razak, Mr. Azmi Abdul Rashid and Dr. Zakaria Wahab for their kind help, valuable suggestions and advice.

Heartiest appreciation is due to Mr. Agus Sutanto, Mr. Ismail Ibrahim and Mr. Ganesan Vadamalai for their kind co-operation during the study period.

I would like to thank Mr. Abd. Rahman b. Sidam, Mr. Daud Mustam, Mr. Shamsuddin Bujang, Mrs. Asiah Othman, Mr. Ahmed, Mr. Bahrin and Mr. Shahril Abd. Rahman for their technical assistance during the period of study.

I am thankful to all my friends, Dr. Matiur Rahman, Dr. Ibrahim Khalil, Dr. Abdul Latif, Dr. A. K. M. Mohiuddin, Dr. Mizanur Rahman, Mr. Abdul Baset, Mr. Golam Farook, Mr. Biplob, Mr. Abu Hena, Mr. Sobi, Mr. Tareque, Mr. Titu, Mr. Swpan, Mr. Mohsin, Mr. Wira Karnain Bin Sani, Mr. Wong Chee Ching, Mr. Hendry, Mr. David, Mr. Franklin, Mr. Philip, Mrs. Nor Shila, Miss. Nor Hayaty, Mrs. Rohaiza, Miss. Nor Hasriah, Mrs. Nor-E-Zam, Mrs. Mazni, Mrs. Nor Ashikin, Mrs. Nor Sheeda, Mr. Zaki, Miss. Jong, Miss. Ng, Miss. Goh, Miss. Chan, Miss. Beverlien, Miss. Aini, Mr. Alexen, Mr. Budi, Mrs. Lia, Miss. Chow, Miss. Lim, Mrs. Deswina, Mrs. Che Raziah, Mrs. Jana, Mr. Aziz, Miss. Bebe, Miss. CY, Mr. Tee, Mr. Sree, Mr. Sobri, Miss. Ana, Miss. Zurida, Mrs. Suzita, Miss. Ramani, Mrs. Rokeya, Mr. Yavuz and Mr. Serkan for their encouragement and always making all things easier and more enjoyable for me.

I am deeply indebted to my loving grand-mother Begum Rokeya, mother Hosne ara Khan, uncle M. Moyen uddin Khan, Dr. Feroz Khan, Mr. Shibli Russel, Mr. Jan Mohd. and Dr. Mohd. Kassim, aunty Dr. Rahila Khanom, Mrs. Rausan ara Khan, Shamim ara Khan and Mrs. Hasinah, cousin Zakir, Razin, Ridwan, Suhaila, Zainura and Jihan for their continuous inspiration, love, understanding and prayers for the success of my study.

Finally, thanks to all my friends who rendered their help in one way or another towards the completion of this study.

I certify that an Examination Committee met on 16th April 2002 to conduct the final examination of Md. Humayun Kabir on his Doctor of Philosophy thesis entitled "Somatic Embryogenesis in *Musa* spp." in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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The thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.

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DECLARATION

I hereby declare that the thesis is based on my original work except for equations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

Md. Humayun Kabir

Date: 6/6/2002

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